Diagnosing intramammary infections: Evaluating expert opinions on the definition of intramammary infection using conjoint analysis

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Diagnosing intramammary infections: Evaluating expert opinions on the definition of intramammary infection using conjoint analysis

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ABSTRACT

The primary purpose of this study was to develop a set of criteria to serve as a pseudo-gold standard for what constitutes an intramammary infection using data from 3 consecutive quarter milk samples taken 1 wk apart. Data from lactating cows in 90 dairy herds in 4 Canadian provinces were used to generate the data sets (profiles) used in the conjoint analysis to elicit expert opinions on the topic. The experts were selected from the participants (n = 23) in the 2007 Mastitis Research Workers' Conference in Minneapolis and from a series of mastitis laboratory courses for bovine practitioners (n = 25) in the Netherlands. Three-week udder quarter profiles with specific combinations of somatic cell count, bacterial species isolated, and plate colony count were selected and included in the conjoint analysis based on the desire to achieve even distributions in the categories of 6 constructed variables. The participants were presented with 3 sets of cards with 20 cards in each set. On each card, they were asked to assign a probability of infection on the middle day (test day) in the 3-wk profile. Depending on the set of cards, they were asked only to be concerned with the probability of infection with coagulase-negative staphylococci, *Escherichia coli*, or *Staphylococcus aureus*. These 3 organisms were chosen to represent a minor pathogen, a major environmental pathogen, and a major contagious pathogen, respectively. The assigned probabilities for each organism were cross-tabulated according to the number of times the organism of interest was isolated in the 3-wk period, how many colonies of the organism of interest were isolated on the test day, and the somatic cell count ($\leq$ or >200,000 cells/mL). There was considerable variation in the assigned probabilities within each of the combinations of factors. The median, minimum, and maximum values of the assigned probabilities for each combination were computed. The combinations with a median probability >50% were considered intramammary infection-positive and included as a criterion in the consensus standard. This yielded 4 possible criteria, which were condensed to the following 2 by consensus at the 2008 Mastitis Research Workers' Conference in Toronto: 1) the organism of interest was isolated on the test day with at least 10 colonies (1,000 cfu/mL), and 2) the organism of interest was isolated at least twice in the 3-wk period.
Key words: intramammary infection, definition, conjoint analysis, gold standard

INTRODUCTION

Mastitis is one of the most important diseases in dairy production, causing substantial economic losses to the industry worldwide. The primary pathway for these losses is the decrease in milk production, mainly caused by subclinical mastitis, making up an estimated two-thirds of the total annual loss caused by mastitis (Bramley et al., 1996).

There is a large volume of literature in which IMI has been defined for different purposes. However, the terminology is not always consistent. Notably, the terms IMI and subclinical mastitis are used almost interchangeably (Barkema et al., 1997; Deluyker et al., 2005). Intramammary infection entails presence of an infectious organism (Berry and Meaney, 2006). The definition is sometimes augmented with a requirement for an increased SCC. Subclinical mastitis indicates inflammation but not necessarily infection of the udder (International Dairy Federation, 1987); however, subclinical mastitis is most often caused by a bacterial infection (Djabri et al., 2002) and this may explain the frequent use of the term subclinical mastitis when referring to an IMI.

Definitions

In a selective review of the recent literature, several definitions of IMI were identified. These typically varied

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[2]Participants at Mastitis Research Workers' Conferences of 2007 and 2008 are recognized for their contributions to the process and their endorsement of the consensus views expressed in this manuscript. A complete list of participants is included in the Appendix.

with respect to the number of samples used to determine IMI status whether an indication of inflammation (usually SCC) was required (and the upper-limit SCC that differentiated a healthy quarter from an infected), the number of organisms cultured, and the number of colonies of the organisms cultured. Single, duplicate, and triplicate quarter milk samples over various time periods have been used to determine IMI status (Dingwell et al., 2003; Bansal et al., 2005; Hillerton et al., 2007).

In papers published in the last 5 yr, the SCC used as a cut point (i.e., minimal value required for a positive classification) varied between 100,000 and 300,000 cells/mL (Schukken et al., 2003; Bansal et al., 2005; Deluyker et al., 2005). With respect to the number of organisms cultured in the samples, some researchers considered a sample contaminated if 3 or more species were cultured (Parker et al., 2008) and others did not make any restrictions to the number of bacterial species cultured (Berry and Meaney, 2006). Several of the reviewed papers used the NMC (1987) guidelines for diagnosing a quarter as IMI-positive or IMI-negative as reference. These guidelines base the confidence of diagnosis on the following criteria: purity of culture (pure, mixed 2 types, mixed several types) and number of colonies isolated (1, several, more than 10). Only 1 of the reviewed papers published during the last 10 yr made use of a minimum colony count for mastitis pathogens: Zadoks et al. (2001) used a minimum colony count of 1,000 cfu/mL when using single samples to determine infection status with *Streptococcus uberis*.

Objectives

This study was conducted as the initial step in a 2-part process with the overall goal of determining the operating characteristics of various definitions of IMI. The second step was to use the consensus standard derived from this study to determine the operating characteristics of the definition of an IMI based on a single quarter milk sample.

The primary objective of this study was to develop a set of rules for classifying the infection status of an udder quarter based on 3 consecutive weekly tests using information about the organism(s) isolated, the number of colonies cultured, and the SCC on each of the 3 test days. To do this, we wanted to identify the factors and the levels of these factors most consistently used by mastitis experts to determine whether a quarter is IMI-positive. This set of rules would serve as the standard for the next part of the research process.

In addition, we documented the level of agreement with regard to the definition of an IMI among mastitis experts, both researchers in the mastitis field and bovine practitioners involved with udder health work.

MATERIALS AND METHODS

Conjoint Analysis

Conjoint analysis is a survey tool commonly used in marketing analysis that originated in mathematical psychology (Luce and Tukey, 1964). A conjoint analysis is often carried out before launching a new product or changing the price of an existing product to determine what factors influence consumer preference. The big advantage of the method is the opportunity to present the survey respondent with constructed combinations of several factors (e.g., price, color, and gas mileage of a new car model) that might influence consumer choice, and the analysis of the responses determines which factors are important in the consumer decision (Cattin and Wittink, 1982). The scenarios in a conjoint analysis survey will typically be a series of theoretical products displaying different levels of the key attributes to be analyzed. Another feature of conjoint analysis is the ability of the method to take interaction between factors into account. This puts the respondent in a situation that simulates the decision making process taking place in real life, in contrast to surveys in which the preference among levels of a single factor is the outcome. The conjoint analysis is carried out by asking the respondents to rank the items with different factor combinations presented to them. The process requires the respondents to make a series of trade-offs when doing so. These trade-offs can be analyzed to reveal the importance of the factors involved (Armstrong, 2001). Thus, the preferences of the respondents are revealed by their selection rather than direct statements about preference of a specific level of a single factor (Churchill, 1999).

Data for Conjoint Analysis

Profiles consisting of organism (a mastitis pathogen), colony count, and SCC for each of 3 weekly samples from a single udder quarter were generated. Three different mastitis pathogens were chosen for the profiles included in the conjoint analysis: *Staphylococcus aureus* represented a major contagious pathogen, *Escherichia coli* represented a major environmental pathogen, and coagulase-negative staphylococci represented a minor pathogen.

Three sets of 20 profiles, 1 for each of the 3 different pathogens, were prepared. Each profile showed information about the organism isolated, colony count, and the

Table 1. Variables used for selection of profiles to be included in the conjoint analysis[1]

Selection variable	Categories
SCC on test day (cells/mL)	<200,000, 200,000–300,000, >300,000
Organism isolated on test day	No growth, present, other organism
Colony count on test day	0, 1–9, ≥10
No. of times organism of interest isolated over 3 samples	0, 1, 2, 3
No. of times SCC >300,000 cells/mL over 3 samples	0, 1, 2, 3
No. of times with maximum colony count (>10 colonies)	0, 1, 2, 3

[1]The goal was to have at least 3 profiles per pathogen (coagulase-negative staphylococci, *Escherichia coli*, and *Staphylococcus aureus*) in every category.

SCC for 3 consecutive weekly tests and was presented on a card for easier ranking of profiles (Figure 1). Two profiles in each set were selected so that the probability of infection was 0 and 100%, respectively. These 2 profiles served as bookends and the respondents were asked to rank the 18 cards in between according to the likelihood that the quarter was infected with the pathogen of interest on the middle of the 3 test days. They were also asked to assign a probability (%) of the quarter being infected on that middle day. Profiles were purposively selected from the available data set so that we had at least 3 profiles in each category of the variables listed in Table 1.

Lactation Quarter Data

The data used to construct the profiles were collected as part of the data collection effort of the National Cohort of Dairy Farms run by the Canadian Bovine Mastitis Research Network, Saint-Hyacinthe, Quebec, Canada (Reyher et al., in press). In this study, quarter milk samples from apparently healthy, lactating cows (n = 15/herd) were taken during the summer of 2007, with the 5 most recently calved cows and 10 randomly selected cows being sampled in each of the 90 participating dairy farms in Alberta, Ontario, Quebec, and the Atlantic provinces. This set of cows was then sampled once weekly for 7 wk. Milk samples were cultured and pathogens were identified according to NMC (1999) guidelines. The respondents were familiarized with the data used to create the profiles through presentations before conducting the conjoint analysis.

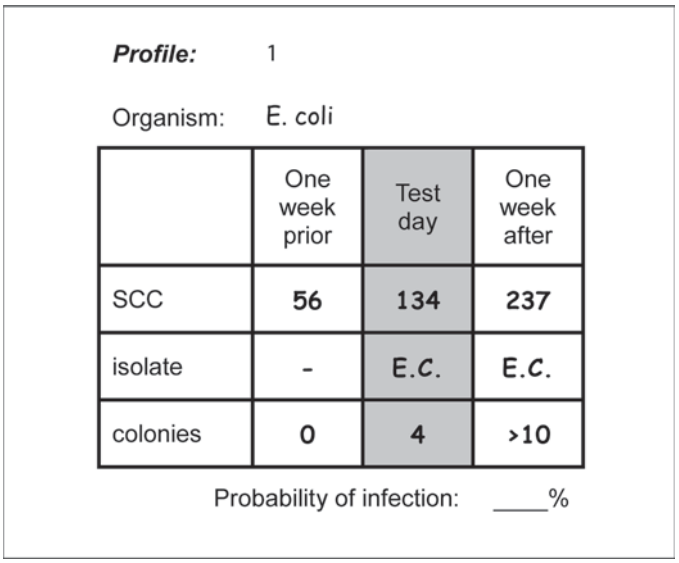

Figure 1. An example of a profile card. The respondents ranked the cards according to the probability of the quarter being infected with the organism of interest (in this case, *Escherichia coli*) on the test day and then assigned a value to that probability.

Respondents

Two locations and sets of respondents were used in the conjoint analysis: participants at the Mastitis Research Workers' Conference (**MRWC**) in 2007 and 2008 (see Table A1 in the Appendix) and bovine practitioners and lay assistants in the Netherlands.

The first phase was conducted at the MRWC in Minneapolis, November 2007, where all participants at the meeting were invited to participate in the analysis. The participants were later classified as "diagnosis experts" or not, with experts having at least 2 yr experience making decisions about infection status of milk samples. The classification of participants was carried out independently by 3 researchers with overlapping knowledge of the participants. If a participant was identified as a diagnosis expert by at least 1 of the classifiers, the participant was considered an expert and their data were included in the analysis. The data from MRWC came from 35 respondents, of which 23 were classified as diagnosis experts.

The second phase of the conjoint analysis was carried out in May and June of 2008 during a series of mastitis laboratory courses for bovine practitioners and lay assistants in the Netherlands. All participants at the courses were invited to participate. Only responses from the bovine practitioners were used in the data analysis. The data from the Netherlands came from 46 respondents, of which 25 were bovine practitioners.

In November 2008, the participants at the MRWC in Toronto had an opportunity to review the classification rules developed as described above. Approximately half of the reviewers were respondents in the survey the previous year (19/35). The classification rules were explained and a table summarizing the results from both locations with all organisms pooled was handed out to all meeting participants interested in reviewing the rules. The participants were asked to circle all rules that they agreed with and cross out rules they did not agree with. Rules for which there was substantial disagreement (a minimum of 5 respondents disagreeing with the rule) were brought forward for discussion. A consensus decision on those rules was obtained from the meeting.

Conduct of Conjoint Analysis

The purpose of the study, the method of construction of profiles, and how to rank the profiles and assign a numeric probability of infection was explained in a 30-min presentation at MRWC in 2007. All participants were given 3 sets of cards, 1 for each organism of interest. They were asked to assign a numerical probability of infection on the middle test day to each card and arrange each set of cards in increasing order of probability of infection according to their estimation of the probability of infection.

Data were computerized and summarized immediately after the survey. The following day, participants were given written feedback with their assigned probabilities of infection for all profiles along with the profile data and the minimum, maximum, and median values for all respondents. They were then given an opportunity to revise their probabilities with this added information. Revised scores were used in all subsequent analyses. The process was repeated at the mastitis laboratory courses conducted in the Netherlands.

Statistical Analysis

Summary statistics such as minimum, maximum, mean, and median values were calculated for each profile. All analyses were carried out using Stata 10 (StataCorp, College Station, TX).

The rules used to classify the profiles as infected or not infected on the test day were derived from cross-tabulations of the number of colonies of the organism of interest (4 categories), the number of times during the 3 wk the organism of interest was isolated (0–3), and the SCC (dichotomized into high and low, with a cut-off value of 200,000 cells/mL) on the test day. For every combination of these factors, the minimum, median, and maximum probabilities assigned by participants were determined. The median was chosen instead of the mean to minimize the effect of skewness in the distributions for each combination. Combinations arising from the cross-tabulations where the median was $\geq$50% were initially considered IMI-positive. The levels of the factors of such a combination constituted a rule for classifying a sample as positive. The classification rules were developed separately for each of the 3 organisms and the 2 locations.

The variation among respondents and within 1 organism and 1 location (MRWC and the Netherlands) of interest was quantified initially by evaluating the range of responses and subsequently through the use of a two-level random effects model. In this model, the variation of the probability of infection was divided into variance components with respondent as random effect and profile as fixed effect. Because of the nonnormal distribution of residuals, the probability of infection was transformed using the inverse sine square root transformation

$$Y_{ij} = u_i + \beta_j + \varepsilon_{ij},$$

where Y_{ij} was the inverse sine square root of the response from the ith respondent for the jth profile, u_i was the random effect of the ith respondent, β_j was the fixed effect of the jth profile, and ε_{ij} was the residual error term in the model. The model was fit using restricted maximum likelihood. The model was run separately for the 3 organisms of interest and the 2 respondent populations (i.e., 6 times in total). The model was fit using restricted maximum likelihood. The model was run separately for the 3 organisms of interest and the 2 respondent populations (i.e., 6 times in total).

RESULTS

Descriptive Statistics

There was a difference in the tendency to accept the opportunity given to revise the probability scores when the participants were provided with the summarized data. At MRWC in 2007, 17% of the responses were revised whereas 40% of the responses from the Dutch bovine practitioners were revised. Revised values were used in all subsequent analyses.

Median values and ranges of the assigned probability of infection for the 3 organisms in the 2 respondent populations are presented in Table 2. These values were derived from the cross-tabulation of 3 variables: number of times the organism of interest was isolated out of 3 wk tabulated against number of colonies isolated on

Table 2. Medians and ranges of the revised assigned probabilities of infection for the 2 respondent populations and across the possible combinations of factors used to determine the rules for an IMI-positive udder quarter[1]

No. of colonies of organism of interest on test day	No. of times organism of interest was isolated in 3 wk	SCC	Mastitis Research Workers' Conference		Dutch bovine practitioners		Combined	
			Median	Range	Median	Range	Median	Range
0	0	Low	0	0–20	2	0–25	1	0–25
		High	5	0–50	5	0–99	5	0–99
	1	Low	4	0–50	10	0–65	5	0–65
		High	15	0–40	19	2–70	15	0–70
	2	Low	20	0–90	37	2–90	30	0–90
		High	45	15–100	43	5–80	43	5–100
1–9	1	Low	20	0–75	20	2–70	24	0–75
		High	22	0–95	20	0–100	20	0–100
	2	Low	60	0–90	80	10–100	80	0–90
		High	—	—	—	—	—	—
	3	Low	—	—	—	—	—	—
		High	95	0–100	90	40–100	95	0–100
≥10	1	Low	50	0–95	70	7–100	60	0–100
		High	76	0–100	80	9–100	80	0–100
	2	Low	85	0–100	80	20–100	80	0–100
		High	—	—	—	—	—	—
	3	Low	90	0–100	90	40–100	90	0–100
		High	90	50–100	95	70–100	92	50–100
Other organism isolated	1	Low	5	0–75	10	2–70	10	0–75
		High	15	0–100	20	0–75	20	0–100
	2	Low	25	0–100	30	0–90	30	0–100
		High	60	0–100	60	5–95	60	0–100

[1]The 3 organisms of interest were combined. The SCC was divided into low and high using 200,000 cells/mL as the cut point. Some combinations have no values because of the absence of profiles in these combinations.

the test day and SCC (dichotomized). As can be seen in Table 2, the results from the 2 respondent populations were very similar.

Median values and ranges for the 2 respondent populations combined across the 3 organisms represented in the profiles are presented in Table 3. They were generally similar, although higher medians for some combinations were observed for *Staphylococcus aureus*.

Level of Variation

It was clear from Tables 2 and 3 that there was a very large range of responses within each profile even though the results (median and range) were quite consistent across populations and organisms. When data for the 3 organisms and the 2 populations were combined (Table 2), more than half of the combinations of factors (11/19) had a range of 95 percentage points or larger.

Even when the responses were stratified according to organism of interest (to account for the organisms' diverse pathobiologies), the ranges were extreme, with 21 of 41 having ranges of 95 percentage points or more. The 1 organism that differed from the values with respect to the medians when the 2 populations were combined was *Staphylococcus aureus*. When the organism of interest was isolated twice in 3 wk but another organism was present on the test day, the median probability of infection for *Staphylococcus aureus* was above 50% regardless of SCC; this was not the case for *Escherichia coli* and coagulase-negative staphylococci. A rule specific for *Staphylococcus aureus* was added to the original set of classification rules to accommodate this difference. The variance estimates from the random effects linear regression analysis are presented in Table 4.

With the variation between profiles removed (by adding profile as a fixed effect), approximately 10 to 30% of the variation was attributable to the respondent. There was a tendency toward slightly less variation among the Dutch bovine practitioners (approximately 10–20%) and more variation among the MRWC participants (approximately 20–30%).

Classification Rules

Using the cross-tabulation method described in Materials and Methods, the initial set of rules for classification of an udder quarter as IMI-positive was as follows:

1. the organism of interest was isolated on the test day with at least 1,000 cfu/mL, or
2. the organism of interest was isolated on the test day and at least 1 other day, or
3. the organism of interest was isolated at least twice in the 3-wk period, and SCC on test day was >200,000 cells/mL, or

Table 3. Medians and ranges of assigned probabilities of infection for the 3 organisms used in the analysis and across the possible combinations of factors used to determine the rules for an IMI-positive udder quarter[1]

No. of colonies of organism of interest on test day	No. of times organism of interest was isolated in 3 wk	SCC	Coagulase-negative staphylococci		*Escherichia coli*		*Staphylococcus aureus*	
			Median	Range	Median	Range	Median	Range
0	0	Low	—	—	0	0–20	2	0–25
		High	5	0–40	5	0–99	8	0–50
	1	Low	—	—	6	0–50	5	0–65
		High	15	0–70	—	—	—	—
	2	Low	—	—	20	0–90	40	5–90
		High	—	—	43	5–100	—	—
1–9	1	Low	20	0–75	—	—	—	—
		High	18	0–95	20	0–90	35	1–100
	2	Low	—	—	80	0–100	—	—
		High	—	—	—	—	—	—
	3	Low	—	—	—	—	—	—
		High	90	0–100	—	—	95	65–100
≥10	1	Low	60	0–100	50	5–100	70	1–100
		High	80	0–100	76	0–100	80	1–100
	2	Low	73	0–100	—	—	90	20–100
		High	—	—	—	—	—	—
	3	Low	85	0–100	90	1–100	94	4–100
		High	—	—	92	50–100	—	—
Other organism	1	Low	7	0–40	—	—	10	0–75
		High	15	0–75	15	0–55	30	0–100
	2	Low	25	0–86	15	0–100	60	3–99
		High	50	0–95	50	2–94	73	10–100

[1]The 2 respondent locations were combined. The SCC on the test day was divided into low and high, using 200,000 cells/mL as the cut point. Some combinations have no values because of the absence of profiles in these combinations.

4. for *Staphylococcus aureus*, the organism was isolated at least twice in the 3-wk period (regardless of SCC).

Revision of Classification Rules

The agreement at the MRWC 2008 meeting was that the consensus standard classification rules be simplified to the following 2 rules:

1. the organism of interest was isolated on the test day with 1,000 cfu/mL or more, or
2. the organism of interest was isolated at least twice out of 3 consecutive weekly tests.

This simplification was further supported by the fact that when the probability of infection was dichotomized with a cut point of 50%, 329 of the 623 responses (53%) for the 2 specific rules (numbers 3 and 4) in the previous section were above the cut point.

Using the Consensus Standard

After establishing a consensus standard, the criteria were applied to the extensive data set from the National Cohort of Dairy Farms summer 2007 sampling of lactating cows from the summer of 2007. The data set consisted of data from 90 herds with a total of 1,351 cows and 6,732 quarters. Although there were a few missing samples from quarters enrolled, the 7 wk of sampling yielded 25,915 observations, each consisting of 3 consecutive weekly milk samples within the same quarter. The classification of quarters according to the

Table 4. The variability of the revised probability of infection for the 2 respondent populations and the 3 organisms used in the conjoint analysis divided into variance components

Respondent population	Organism of interest	Respondent variation (% of total variation)	Residual variation (% of total variation)
Mastitis Research Workers' Conference	Coagulase-negative staphylococci	31	69
	Escherichia coli	19	81
	Staphylococcus aureus	26	74
Dutch bovine practitioners	Coagulase-negative staphylococci	20	80
	Escherichia coli	9	91
	Staphylococcus aureus	21	79

Table 5. Classification of the 3 organisms used in the conjoint analysis according to the final consensus standard classification criteria[1] (no. of observations = 25,915)

Consensus standard status	Organism of interest isolated at least twice in 3 wk	No. of colonies of organism of interest isolated on test day	Organism of interest		
			Coagulase-negative staphylococci	*Escherichia coli*	*Staphylococcus aureus*
Negative	No	Negative or other organism	3,679	29	112
	No	1–9	1,671	7	20
	Total consensus standard negative		5,350 (64%)	36 (78%)	132 (26%)
Positive	No	≥10	156	4	20
	Yes	Negative or other organism	638	2	46
	Yes	1–9	1,236	3	47
	Yes	≥10	985	1	258
	Total consensus standard positive		3,015 (36%)	10 (22%)	371 (74%)
Overall total with organism of interest present at least once			8,365	46	503

[1]Consensus standard rules: 1) the organism of interest was isolated on the test day with 10 colonies or more, and 2) the organism of interest was isolated at least twice out of 3 consecutive weekly tests.

factors in the tables, from which the consensus standard is derived, as well as the consensus standard itself, is displayed in Table 5.

The vast majority of quarters did not have the organism of interest isolated on the test day (top row of Table 5). For coagulase-negative staphylococci, 20% of the time when coagulase-negative staphylococci were isolated on the middle test day, the quarter was considered IMI-negative according to the consensus standard. The same was true for 4% of *Staphylococcus aureus* and 15% of *Escherichia coli* cases.

DISCUSSION

Importance

The frequent quarter milk samples collected from a random selection of apparently healthy, lactating cows in a wide variety of herds in 4 regions of Canada presented a unique opportunity to acquire culture and SCC data on quarter level and on a regular basis for an extended period of time from a large number of quarters.

The need for a common definition of IMI resulted from a desire to homogenize the definition used in the wide range of research projects within the Canadian Bovine Mastitis Research Network as well as by independent researchers in related fields. A common defensible definition based on a thorough evaluation of existing definitions of IMI would facilitate comparison of studies in the mastitis field and thereby make results more accessible.

The development of a true gold standard for the IMI status of an udder quarter would involve invasive monitoring procedures and would be neither feasible nor ethically defensible. Consequently, the conjoint analysis was used to provide a basis for the development of a consensus standard for IMI status of a quarter.

It was important to distinguish between IMI and mastitis, the first term implying only the presence of a pathogen and the latter only the presence of an inflammatory response. This study concerned only IMI; even so, several respondents indicated that SCC was a factor in making a decision about the probability of infection of the quarters presented in the conjoint analysis. For instance, ranges of probability of infection for both populations combined when the organism of interest was isolated 3 times in 3 wk with 10 or more colonies changed from 0 to 100% when SCC was low (≤200,000 cells/mL) to 50 to 100% when SCC was high (>200,000 cells/mL). Similarly, when the organism of interest was not isolated at all, the range changed from 0 to 25% in the low SCC category to 0 to 99% in the high SCC category. The importance of SCC was also reflected in the initial set of classification rules, which included a rule stating that the quarter would be deemed infected if the organism of interest was isolated twice in 3 wk, but only if the SCC on the test day was increased (>200,000 cells/mL). At MRWC 2008, this rule was simplified and the SCC condition was removed with the argument that the definition concerned only IMI, not mastitis, and therefore an indicator of inflammatory response was not necessary.

Classification Rules Based on Descriptive Statistics

A large degree of variation in the assigned probabilities of infection was observed among the respondents even though they had a chance to revise their scores

with summary statistics for all profiles on hand. There was a prior expectation of some degree of variation among experts, but the variation observed in the conjoint analysis was surprisingly large. The revision process was inspired by the Delphi technique (van Zolingen and Klaassen, 2003), in which survey subjects are given multiple opportunities to revise their answers, knowing the responses of the other subjects. This technique will often limit the distribution of responses because subjects are influenced by the response of other respondents (van Zolingen and Klaassen, 2003). Apparently, beliefs about probability of intramammary infection are firmly held among mastitis experts, even if severely at odds with consensus opinion, because relatively few probabilities were revised. This tendency was less apparent among the Dutch bovine practitioners.

When the level of variation was quantified, a considerable amount (10–30%) of the random variation observed could be explained by the variation between respondents. This variation between respondents was most likely attributable to a tendency among some respondents to systematically assign higher or lower probabilities of infection to their profiles when the conjoint analysis was conducted.

The similarity in responses among the 2 groups of respondents, the mastitis researchers and the Dutch bovine practitioners, was reassuring. The classification rules derived from the median values of probability of infection would have been identical had they been derived from the 2 populations separately. Data from the respondent populations were therefore combined to simplify presentation.

The similarities among the 3 organisms of interest were more surprising because of the selection of organisms based on their diverse routes of transmission and infection. Only *Staphylococcus aureus* differed enough that a specific classification rule was added initially. This rule, however, was extended to apply to all organisms in the revision of the classification rules at MRWC 2008.

Multivariable linear regression was attempted (not shown) to quantify the effect of factors used when classifying a quarter as infected or not. However, because of the inability to generate stable plausible models regardless of how the predictors were modified (e.g., coding of continuous predictors), this approach was abandoned in favor of basic statistical methods and tabular displays of selected factors. The method used could potentially be improved by ensuring 1 or more profiles within each combination of the factors and organism. This process, however, was somewhat limited by what scenarios occurred in the data set and the limited time available between the data collection and the first phase of the conjoint analysis. Another way of improving our confidence in the estimates of medians and potentially limiting the ranges of the assigned probabilities would be to use a larger number of respondents. However, this was not logistically feasible.

Using the Consensus Standard

The differences among the organisms of interest manifested itself in the fractions of quarters that were positive for the organism on the test day but were considered IMI-negative according to the consensus standard. *Staphylococcus aureus* differed from both *Escherichia coli* and coagulase-negative staphylococci by having a considerably lower percentage (4% vs. 15 and 20%, respectively) of these cases. This could be explained by the tendency of *Staphylococcus aureus* to either not occur at all in a quarter during a 3-wk period or occur 2 or more times (often in large numbers). The next step in the process of defining IMI will be to evaluate a range of single sample definitions using the same data used in this study and the consensus standard for 3 consecutive samples.

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APPENDIX

Participants at the MRWC meetings. Participants (n = 5) who did not respond to the written request for consent to be included as coauthors were not included.

Table A1. Participants at the 2007 and 2008 Mastitis Research Workers' Conference (MRWC) meetings

Name	Participant MRWC (2007)	Participant MRWC (2008)
Andersen, Signe	Yes	Yes
Barkema, Herman	Yes	Yes
Barlow, John	Yes	Yes
Calloway, Chris	No	Yes
De Vliegher, Sarne	Yes	Yes
Dohoo, Ian	Yes	Yes
Dufour, Simon	Yes	Yes
Erskine, Ron	Yes	No
Fox, Larry	Yes	Yes
Hulland, Carol	Yes	Yes
Keefe, Greg	Yes	Yes
Lago, Alfonso	Yes	No
Leslie, Ken	Yes	No
Lichtenwalner, Anne	No	Yes
MacDonald, Kimberley	No	Yes
McClure, J. T.	No	Yes
McDougall, Scott	Yes	No
Middleton, John	Yes	Yes
Mullarky, Isis	No	Yes
Owens, William	Yes	Yes
Perez-Casal, Jose	No	Yes
Piepers, Sofie	No	Yes
Reyher, Kristen	Yes	Yes
Riekerink, Richard Olde	Yes	Yes
Roy, Jean-Philippe	No	Yes
Scholl, Daniel	Yes	Yes
Schukken, Ynte	No	Yes
Ster, Celine	No	Yes
Supre, Karlien	No	Yes
Wenz, John	No	Yes
Wilson, David	No	Yes
Zadoks, Ruth	Yes	Yes